ONE STEP RT-PCR METHODS, ENZYME MIXES AND KITS FOR USE IN PRACTICING THE SAME

ABSTRACT

Enzyme compositions, kits comprising the same and methods for their use in one-step RT-PCR are provided. The subject enzyme compositions at least include a mutant thermostable DNA polymerase and a mutant reverse transcriptase. In preferred embodiments, the mutant thermostable DNA polymerase is an N-terminal deletion mutant of Taq polymerase and the mutant reverse transcriptase is a point mutation mutant of MMLV-RT. The subject kits, in addition to the above described mutant thermostable DNA polymerase and mutant reverse transcriptase, at least include one of, and usually both of, dNTPs and a buffer composition, where the subject kits may further include additional reagents, including nucleic acids, a thermostabilizing agent, a glycine based osmolyte and the like. In practicing the subject methods, a reaction mix that at least includes template RNA, the above described mutant polymerase and reverse transcriptase, dNTPs, buffer, and nucleic acid primers is prepared. The resultant reaction mixture is maintained at a first set of reverse transcription conditions and then a second set of PCR conditions, whereby amplified amounts of DNA from a template RNA(s) are produced.